Sub D4 74. (New) A method of enhancing the therapeutic effect of a foreign therapeutic gene administered to a patient, said method comprising the steps of:

(a) administering a cell cycle blocking agent to said patient; and

(b) administering said foreign therapeutic gene to said patient within seven days of step (a), wherein said foreign therapeutic gene is administered in a lipid formulation comprising a cationic lipid and a lipid selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid.

C3

75. (New) The method of claim 74, wherein said (PEG)-lipid derivative is a PEG-ceramide.

76. (New) The method of claim 75, wherein said PEG-ceramide is a member selected from the group of PEG-Cer-C14, PEG-Cer-C20, and PEG-Cer-C8.

Sub D5 77. (New) The method of claim 74, wherein said lipid is present in an amount of from about 1% to about 20%.

REMARKS

I. The Invention

The present invention relates to methods for increasing the efficiency of the transfection of cycling cells. The methods involve synchronizing cells at a first stage of the cell cycle, and transforming the cells at a second stage of the cell cycle within about one cell cycle of the first stage with a nucleic acid. The invention further relates to cancer therapy and, in particular, to methods of efficiently transfecting cancer cells with nucleic acids.

II. Status of the Claims

Claims 1-55 are pending. Claims 1-55 were examined and stand rejected. Applicants have canceled claims 1-37 without prejudice or disclaimer. Applicants have amended claims 38 and 55. Claims 56-77 have been added. Applicants believe the present amendment does not introduce any new matter and respectfully request reconsideration and reexamination of claims 38-77. All of the amended and pending claims are reproduced in an attached Appendix as they will appear upon entry of the present amendment.

III. Objection to claim 35

The Examiner objected to claim 35 for the misspelling of nucleic acid.

Applicants have canceled claim 35. Applicants respectfully request that the objection be withdrawn.

IV. RejectionUnder 35 U.S.C. § 112, First Paragraph

Claims 1-55 were rejected under 35 U.S.C. § 112 first paragraph, for lack of enablement. Briefly, the Examiner alleges that there is insufficient guidance in the specification to practice the claimed invention in light of the breadth of the claims and the alleged unpredictability of the gene therapy arts (e.g., particularly targeting of genes to appropriate tissues, gene constructs for delivery, routes of administration, gene expression levels, and therapeutic dosages of a tumor inhibitory gene and a cell cycle synchronizer).

Applicants respectfully traverse this rejection and respectfully disagree with the contentions in the Office Action as to this rejection. As identified by the Patent Office and the Federal Circuit, the proper standard for determining whether undue experimentation is required by one skilled in the art to practice the invention includes consideration of factors such as the amount of guidance provided in the application and the presence of working examples. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int. 1985); *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). Furthermore, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should precede." *Wands*, 8 USPQ2d at 1404 (quoting *In re Jackson*, 217 USPQ 804 (Bd. Pat. App. & Int. 1982). Also, it is important to note that the possibility that some experimentation, even if such experimentation is complex or extensive, may be required for the practice of the invention does not necessarily mean that the invention is not enabled:

> [t]he fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *See*, MPEP § 2164.01.

Applicants contend the claimed invention can be practiced without undue experimentation. Applicants have amended claims. In the Office Action, the Examiner referred to several factors contributing to the conclusion that the claims were not enabled: (1)

the relevance of mouse models, (2) administration of the gene delivery vehicle, (3) targeting of the DNA vehicle, and (4) gene transfer efficiency. These factors will be discussed in turn.

1. The relevance of mouse models

The Examiner cited Orkin as supporting the importance of relevant animal models for determination of the effectiveness of gene therapy treatment. The Examiner quoted Orkin as saying "many mouse models do not faithfully mimic the relevant human conditions" and that animal models are less predictive when applied to cancer.

Applicants respectfully submit that the extensive guidance provided in the specification and the presence of working examples in an animal model support the conclusion that the claimed invention is enabled. For example, the specification discloses that using the methods of the claimed invention, solid tumors can be reduced with the intravenous administration of vincristine sulfate and a plasmid encoding IL-12 (page 54, lines 1-26). In addition, tumors can be reduced using intravenous injections of vincristine sulfate, a nucleic acid encoding thymidine kinase, and ganciclovir (page 54, line 27, bridging to page 55, line 31).

Moreover, the reference that the Examiner cites, Orkin, emphasizes the value of animal models in leading to successful gene therapy approaches, rather than dismissing the use of animal models in human gene therapy. For example, Orkin states that "the study of animal models for the design of gene therapy approaches in a preclinical setting is important and should not be undervalued." (See page 11, paragraph four).

In addition, a patent cited by the Examiner as 35 U.S.C. § 102(e) art, Roth *et al.* (U.S. Patent 5,7474,469) (see anticipation rejection below), contains a claim drawn to gene therapy and only discloses mouse model data in the specification. Claim 1 of Roth *et al.* recites:

> 1. A method of killing a tumor in a patient in need thereof, comprising directly administering to said tumor cell therapeutically effective amounts of a viral vector and a DNA damaging agent, wherein said viral vector comprises a DNA sequence encoding p53 operatively linked to a promoter, and wherein expression of said p53 and DNA damage result in the killing of said tumor cell.

Issued patents are considered to contain fully enabling disclosures and are presumed valid under 35 U.S.C. § 282. *See Ex parte Goldgaber*, 41 U.S.P.Q.2d 1172, 1175 (Bd. Pat. App. & Int'f 1995). Thus, claim 1 of Roth *et al.* is directed towards *gene therapy*, for *all tumor types*. Roth *et al.*, however, do not show *human* data. Roth *et al.* describe the use of *mouse*, not human, models. Therefore, it is surprising that the Examiner recites the necessity of human clinical data, when Roth *et al.* do not contain any human clinical data.

Furthermore, Applicants remind the Examiner that the case law is clear that a demonstration of efficacy in experimental animals is more than sufficient to establish utility for broad claims for an invention under 35 U.S.C. § 112. For example, in *In re Jolles*, 206 USPQ 885 (CCPA 1980), the CCPA stated that:

> [t]his court recognizes 'that a demonstration that a compound has desirable or beneficial properties in the prevention, alleviation, or cure of some disease or manifestation of a disease in experimental animals does not necessarily mean that the compound will have the same properties when used with humans.' ***However, this is by no means support for the board's position that such evidence is not relevant to human utility.***
>
> To the contrary, this court has accepted tests on experimental animals as sufficient to establish utility.... *Id.* at 890 (citations omitted; emphasis added).

Thus, the demonstration that the present formulations are effective in mice is an indication that undue experimentation is not required to practice the claimed invention. It is important to note that the Federal Circuit's position in this regard recognizes the essentially universally held position among scientists that work in experimental mammals is of critical importance and relevance to other mammals, including humans. Even a reference cited by the Examiner in this rejection, Crystal (1995), clearly accepts the importance of research in mice, and simply points out that, occasionally predictions based on work in mice do not prove true in humans. As stated in Crystal:

> *[h]umans are not simply large mice.* There have been several ***surprise examples***, in which predictions from gene transfer studies in experimental animals have not been borne out in human safety and efficacy trials. (page 409; non-bold italics in original, bold italics added).

This is entirely consistent with the above-described position of the Federal Circuit, *i.e.*, that work in mice has great predictive value in determining efficacy in other animals, such as humans. The fact that, occasionally, "surprise examples" arise in which these predictions are not borne out, in no way detracts from the value of this work or from the reasonable expectation of success that scientists have in extrapolating from work performed in experimental animals to other animals. Therefore, sufficient guidance is provided in the specification such that undue experimentation is not required to practice the claimed invention.

2. Administration of the gene delivery vehicle.

The Examiner cites Bischoff *et al.* as teaching that the benefits of direct intratumoral injection of gene therapy vectors are limited only to accessible tumors, and that metastatic tumor cells will not be killed by this method. The Examiner further alleges that the effect of any tumor inhibitory gene by any route of administration is unpredictable in the art.

Applicants respectfully disagree with the Examiner's conclusion. Bischoff *et al.* was directed to the effects of intratumoral injection and stated that their method would be limited to intratumoral injections. A close inspection of Bischoff *et al.* reveals that the method is directed towards the treatment of p53 deficient cervical carcinomas (see Abstract). However, the present specification demonstrates that for example, intratumoral administration is effective at targeting expression in the tumor (page 51, line 10, bridging to page 52, line 21). Intravenous administration also resulted in targeting of expression of the nucleic acid in tumors (page 49, line 23, bridging to page 50, line 5; page 54, line 27, bridging to page 55, line 31; page 54, lines 1-26) and spleen cells (page 52, line 23, bridging to page 53, line 30). The specification also notes that the genes can be delivered by two major classes of methods, (1) local or regional delivery (e.g., inhalation, injection, etc.) and (2) systemic delivery (e.g., intravenous) (page 25, lines 2-5). Moreover, techniques for *in vivo* delivery of genes are known to those of skill in the art and are the focus of over 200 FDA approved clinical trials (page 25, lines 8-9). Therefore, the specification provides adequate guidance and examples for one of skill in the art to administer the cell cycle synchronizers and nucleic acids of the claimed invention.

3. Targeting of the DNA vehicle.

The Examiner cites Deonarian for the proposition that "one of the biggest problems hampering successful gene therapy is the ability to target a gene to a significant population of cells and express it for a long enough period of time." The Examiner also cites Dachs *et al.* as reciting that gene therapy is unpredictable because "effective and selective delivery of DNA to tumor cells is a complex task due to a poor and disorganized blood supply, and high interstitial fluid pressure of a solid tumor." The Examiner states vector targeting is unpredictable and inefficient by quoting Miller *et al.* for the conclusion that "for the long term success as well as the widespread applicability of human gene therapy, there will have to be advances...targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems."

Applicants respectfully disagree with the Examiner's conclusions as to the unpredictability of targeting genes using the claimed methods.

Dachs *et al.*

The Examiner cites Dachs *et al.* as pointing out problems with gene targeting. The Examiner, however, has ignored the other statements in Dachs *et al.* that argue for predictability of gene targeting. For example, Dachs *et al.* state that "[c]ancer gene therapy can combine highly specific gene delivery with highly specific gene expression." Dachs *et al.* further report on five successful methods of targeted gene delivery and thirteen successful methods of targeted gene expression (pages 314-318). Furthermore, with regard to delivery of DNA to solid tumors, Dachs *et al.* describe a method of targeted gene expression that takes advantage of the disorganized blood supply and transient low oxygen levels of solid tumors (pages 318-319).

Deonarian

Furthermore, Deonarian actually reviews a particular type of gene therapy targeting system (ligand-targeted, receptor-mediated vectors for gene therapy) and describes this therapy as successful, with a higher level of tissue specificity than viral vectors (*see*, page 53, first paragraph). In addition, Deonarian goes on to say that "[u]nder optimal conditions, enough gene product may be produced to give a therapeutic benefit (e.g. suppress a phenotype or destroy a tumor)." Thus, Deonarian speaks only to the low efficiency of the targeting

technique. Accordingly, Deonarian does not state that targeting gene therapy constructs to particular cells is unpredictable, merely that the technique could be improved and is complex.

Miller *et al.*

Applicants disagree with the Examiner's conclusion as to the import of Miller *et al.* Furthermore, Applicants draw the Examiner's attention to Miller *et al.* where the reference sets out that "it is clear that the technology **now** exists to incorporate specific targeting features into most of the currently available delivery systems." (see page 190; emphasis added). Miller *et al.* go on to state that two targeting strategies can be achieved "at the level of 1) target cell recognition, by recognition components of viruses and liposomes; or 2) target cell transcriptional restrictions" (page 190). Thus, Miller *et al.* arguably disclose that targeting of genes is possible and disclose methods to achieve that end.

Guidance and working examples in the specification

The specification also provides guidance on methods to target DNA to cells. For example, the specification discloses methods to specifically target the composition comprising the DNA to a cell using a targeting moiety (e.g., antibodies, proteins, etc.) to a lipid (page 32, lines 11-14).

In addition, the Examiner ignores the working examples in the specification which demonstrate that cycling cells are targeted with the claimed methods. Again, the invention involves targeting cycling cells with the cell synchronizers of the claimed invention. It is the use of the cell synchronizers that increase the efficiency of introducing a desired nucleic acid into a cycling cell. Thus, cycling cells are targeted by virtue of the nature of the pharmacological activity and specificity of cell synchronizers (or blockers). For example, the specification demonstrates that intravenous injection of tumor bearing-mice with OncoTCS (vincristine sulfate encapsulated in sphingomyelin-containing TCS) followed by intravenous injection with a luciferase-encoding-plasmid resulted in luciferase expression in the tumor (page 49, line 23, bridging to page 50, line 5). Similar results were achieved with OncoTCS and the TK/ganciclovir system with a luciferase-encoding-plasmid (page 54, line 27, bridging to page 55, line 31). Furthermore, spleen cells were also targeted with OncoTCS and a luciferase-encoding-plasmid by intravenous injection (page 52, line 23, bridging to page 53,

line 30). In addition, successful tumor growth inhibition was achieved using intravenous injection of OncoTCS and a plasmid encoding IL-12 (page 54, lines 1-26). Targeting to tumors was also achieved with OncoTCS and a luciferase encoding plasmid to a solid tumor by intratumoral injection (page 51, line 10, bridging to page 52, line 21). These numerous examples demonstrate that the administration of a cell cycle synchronizer is able to increase the efficiency of the nucleic acid transformation of cells and is able to achieve cell targeting.

4. Gene transfer efficiency

The Examiner cited Crystal as saying "among the design hurdles for all vectors are the need to increase the efficiency of gene transfer, to increase specificity and to enable the transferred gene to be regulated."

Applicants point out that, contrary to the Examiner's statements, that Crystal in fact, teaches that gene therapy is a viable method to treat patients and taken as a whole describes gene therapy as an impressive accomplishment. For example, Crystal recites that:

> [p]robably the most remarkable conclusion drawn from the human trials is that human gene transfer is indeed feasible. Although gene transfer has not been demonstrated in all recipients, *most studies have shown that genes can be transferred to humans whether the strategy is ex vivo or in vivo, and that all vector types function as intended.* Taken together, the evidence is *overwhelming*, with successful gene transfer having been demonstrated in 28 ex vivo and *10 in vivo studies*. (page 405) (emphasis added).

> [c]linical experience to date suggests that retrovirus, adenovirus, and plasmid-liposome vectors all need refinement, but each is relatively well suited for the clinical targets at which they have been directed. Further, the technology is now available to create designer vectors that can be optimized for each application. (page 409).

Thus, it is improper to characterize Crystal as demonstrating that gene therapy is unfeasible or too unpredictable for one of skill in the art to use the invention as directed by the specification. In view of the foregoing remarks, Applicants respectfully request that the rejection be withdrawn as the claimed invention can be practiced without undue experimentation.

V. Rejection Under 35 U.S.C. § 112, Second Paragraph

The Examiner rejected claims 1-55 under 35 U.S.C. §112, second paragraph as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which the applicants regard as the invention.

Applicants respectfully traverse this rejection. In the instant case, the specification adequately defines the terms or the terms are adequately understood by one of skill in the art, such that that claims are not indefinite under 35 U.S.C. §112, second paragraph. A determination of claim definiteness under 35 U.S.C. § 112, second paragraph, must be made in view of the (A) content of the application, (B) the teachings of the prior art, and (C) the claim interpretation given by one of ordinary skill in the art. *see* MPEP § 2173.02; *Autogiro v. United States*, 155 USPQ 697 (Ct. Cl. 1967) (The claims are to be read in light of the specification and the Applicant is free to be their own lexicographer); *see also*, MPEP § 2111.01. Several bases of indefiniteness were raised, and they will be discussed in turn.

A. "Cell synchronizer or synchronizes"

Claims 1-37 were allegedly indefinite according to the Examiner because the phrase "cell synchronizer or synchronizes" are allegedly unclear as to whether the cells are stopping in the same phase at the same time or the cells stop within phases.

Applicants have canceled claims 1-37 without prejudice or disclaimer. Accordingly, Applicant respectfully request that this rejection be withdrawn.

B. "Cell cycle blocker"

According to the Examiner, claims 38-55 are allegedly indefinite because the phrase "cell cycle blocker" is unclear as to what factors are encompassed in the claim to determine whether all the cells are blocked at the same time.

Applicants respectfully traverse this rejection. The term cell cycle blocker is defined in the application at page 13, lines 4-10 as a "synchronizing compound or a radiation treatment, such as X-rays, that inhibits a cell from proceeding into a subsequent cell cycle phase to which the cell would proceed in the absence of the compound." Thus, a cell cycle blocker can be used interchangeably with cell synchronizer. As discussed above, synchronizing generally means increasing the fraction of cells that are at a certain stage of the cell cycle. For the above reasons, Applicants respectfully submit that the term "cell cycle blocker" as used in the present claims, would have been entirely clear to one of skill in the art. Accordingly, Applicants respectfully request that the rejection be withdrawn.

C. "Substantially"

According to the Examiner, claims 3 and 16 are allegedly indefinite because the term "substantially" is unclear as to what standard is used to determine if the nuclear membrane is substantially degraded.

Applicants have canceled claims 3 and 16. Accordingly, Applicants respectfully request that the rejection be withdrawn.

VI. Rejection Under 35 U.S.C. § 102(e)

Claims 1-3, 8, 10-14, 15-16, 21, 23-32, 35, 38-44, 46-47, 49, 52, and 55 are rejected under 35 U.S.C. § 102(e) as being anticipated by Roth *et al.* (U.S. Patent 5,7474,469).

Applicants respectfully traverse this rejection. Anticipation under §102 requires that "each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of Calif.*, 814 F.2d 628, 631 (Fed. Cir. 1987). The "exclusion of a claimed element from a prior art reference is enough to negate anticipation by that reference." *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1574 (Fed. Cir. 1984).

Claims 38 and 55 recite that the foreign gene is fully encapsulated in a lipid formulation such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C. Roth et al., however do not teach lipid formulations that satisfy the requirements of this limitation; Roth et al. do not teach any lipid formulations wherein a foreign therapeutic gene is stable to the DNAse treatment set forth in the claims. Therefore, Roth et al. do not anticipate the claimed invention.

Independent claims 56 and 69 recite that the foreign therapeutic gene is administered systemically. However, Roth et al. do not teach formulations of a foreign gene that can successfully be used when delivered systemically. As such, Roth et al. do not disclose operable embodiments that fulfil all the limitations of the claimed invention. The formulations of the p53 gene or protein disclosed in Roth et al. will not work if they are delivered systemically. Therefore, Roth et al. do not anticipate the claimed invention.

Independent claim 74 recite that the foreign therapeutic gene is administered in a lipid formulation comprising a cationic lipid and a lipid selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid. However, Roth et al. fail to disclose formulations of a gene using these compounds. Therefore, Roth et al. fail to teach all of the elements of the claimed invention. Therefore, Roth *et al.* do not disclose all of the elements of the claimed invention. Accordingly, Applicants respectfully request that the rejection be withdrawn.

VII. Rejection Under 35 U.S.C. § 102(b)

Claims 1-3, 8, 15-16, 21, 28, 32-34, 38, 41, 44, 46, 47, 49-51, and 55 were rejected under 35 U.S.C. § 102(b) as being anticipated by Son *et al.*, *Proc. Natl. Acad. Sci.* (1994), 91:12669-12672.

Applicants respectfully traverse this rejection. As stated above, an anticipatory reference must disclose every element of the claimed invention. Son et al., however, do not disclose the use of a foreign therapeutic gene. The gene disclosed in Son et al. is chlorampenicol acetyltransferase (CAT) which is not a therapeutic gene. The CAT gene is an antibiotic resistance gene which would does not produce a "gene product [which} performs a clinically useful function" (See Application, at page 15, line 11-12). Therefore, Son et al. fail to anticipate the claimed invention.

Moreover, independent claims 38 and 55 recite that the foreign gene is fully encapsulated in a lipid formulation such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C. Son et al., however do not teach lipid formulations that satisfy the requirements of this limitation. The cationic liposome formulations of Son et al. are not stable to 1 U DNAse I for 30 minutes in digestion buffer at 37°C. That is why they must be administered directly into the tumor (see page 12669). Therefore, Son et al. do not anticipate the claimed invention.

Independent claims 56 and 69 recite that the foreign therapeutic gene is administered systemically. However, Son et al. do not teach formulations of a foreign gene that can successfully be used when delivered systemically. Son et al. in fact deliver the cationic liposomes by injected them directly into the tumor (see page 12669). Moreover, the formulations of Son et al. if delivered systemically, would break down too rapidly to achieve the claimed invention. Therefore, Son et al. do not anticipate the claimed invention.

Independent claim 74 recite that the foreign therapeutic gene is administered in a lipid formulation comprising a cationic lipid and a lipid selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid. However, Son et al. fail to disclose formulations of a gene using these compounds. Rather, Son et al. use a formulation of a DC-Chol and dioleoyl phosphatidylethanolamine (see page 12669). Therefore, Son et al. fail to anticipate the claimed invention.

In view of the foregoing remarks, Applicants respectfully request that the rejection be withdrawn.

VIII. Rejections Under 35 U.S.C. § 103(a)

A. The claimed invention is nonobvious over Krek *et al.*, in view of Roth *et al.* and Lee *et al.*

The Examiner rejected claims 1, 4-7, 9, 15, 17-20, and 22 under 35 U.S.C. § 103(a) as being obvious over Krek *et al.*, *Methods in Enzymol.* (1995) 254:114-124, in view of Roth *et al.* and Lee *et al.*, *Cancer Res.* (1996) 56:1303-8.

Applicants respectfully traverse this rejection.

Applicants have canceled claims 1-37 without prejudice or disclaimer. In view of the cancellation of those claims, Applicants respectfully request that the rejection be withdrawn.

B. The claimed invention is nonobvious over Son *et al.* and Roth *et al.*

The Examiner rejected claims 15, 36-38, 45, and 53-54 under 35 U.S.C. § 103(a) as being obvious over Son *et al.* in view of Roth, *et al.* Examiner alleged that Roth *et al.* cured the deficiency of Son *et al.*'s failure to disclose that the foreign gene is administered "at least 32 to 48 hours prior to administering the cell cycle blocking agent." The Examiner felt that Roth *et al.* filled the gap in teaching by disclosing that the vector could be administered "prior, after, or at the same time of the DNA damaging agent."

As set forth in M.P.E.P. § 2143, "[t]o establish a *prima facie* case of obviousness, three basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim elements. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

Applicants respectfully traverse this rejection and assert that a *prima facie* case of obviousness has not been made out as Son *et al.* and Roth *et al.* do not contain a suggestion or motivation to modify the references or to combine reference teachings. *Also*, there is no reasonable expectation that the combination that the Examiner contemplates would succeed. *Finally*, Son *et al.* and Roth *et al.* do not teach or suggest all the claim elements.

Independent claims 38 and 55 recite that the foreign gene is fully encapsulated in a lipid formulation such that less than 5% of the gene is degraded after exposure of the formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C. Son et al. and Roth et al., however do not teach lipid formulations that satisfy the requirements of this limitation. The cationic liposome formulations of Son et al. are not stable to 1 U DNAse I for 30 minutes

in digestion buffer at 37°C. Similarly, Roth et al. do not teach any lipid formulations wherein a foreign therapeutic gene is stable to the DNAse treatment set forth in independent claims 38 and 55.

The Examiner alleged that it is "well known in the art of liposomal formulation to encapsulated [sic] DNA within the liposome complex and then to subject the liposomal complex to DNAse treatment was [sic] to remove excess unencapsulated DNA." Applicants respectfully traverse this assertion and request that the Examiner cite a reference in support of this position that it would have been obvious to one of skill in the art to fully encapsulate a gene in a lipid formulation such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C. See MPEP § 2144.03. Applicants maintain that the claimed lipid formulations were not taught in Son et al. as cationic lipid-DNA complexes prepared by the method disclosed therein does not fulfill the claimed limitations. Applicants point out that the DNAse treatment is the test of whether a lipid formulation fulfills this particular claim element and is not the method of preparing the fully encapsulated foreign gene. Thus, even if the Son et al. and Roth et al. references were combined, it would not result in the *claimed* invention.

Independent claims 56 and 69 recite that the foreign therapeutic gene is administered systemically. However, Son et al. and Roth et al. do not teach formulations of a foreign gene that can successfully be used when delivered systemically. Son et al. in fact deliver the cationic liposomes by injecting them directly into the tumor (see page 12669). Moreover, the formulations of Son et al. if delivered systemically, would break down too rapidly to achieve the claimed invention. Similarly, the formulations of the p53 gene or protein disclosed in Roth et al. will not work if they are delivered systemically. Roth et al. do not describe formulations or preparations that can successfully be used when delivered systemically. In point of fact, Roth et al. do not disclose working examples of their methods working when delivery is systemic. Given the unpredictability of a delivery method being able to successfully deliver a gene (see page 5 of the Office Action), one of skill would not have had a *reasonable* expectation that the combination of Son et al. and Roth et al. would result in successfully achieving the claimed invention.

Independent claim 74 recite that the foreign therapeutic gene is administered in a lipid formulation comprising a cationic lipid and a lipid selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid. However, Roth et al. and Son et al. fail to disclose formulations of a gene using these compounds. For example, Son et al. use a formulation of a DC-Chol and dioleoyl phosphatidylethanolamine (see page 12669). Therefore, Roth et al. and Son et al. fail to teach all of the elements of the claimed invention. It follows that there would not be a motivation or suggestion to combine the cited references or a reasonable expectation that the combination would succeed in view of this lack of teaching all of the claimed elements. One of skill could not be motivated to combine references to obtain the claimed invention.

In view of the foregoing remarks, Applicants respectfully request that the rejection be withdrawn.

C. The claimed invention is nonobvious over Son *et al.*, Roth *et al.*, and Walker *et al.*

The Examiner rejected claims 38 and 48 under 35 U.S.C. § 103(a) as being obvious over Son *et al.* in view of Roth *et al.* in further view of Walker *et al.* (U.S. Patent 6,041,252). The Examiner alleged that one of skill would have been motivated to deliver a cell cycle blocker in a liposome formulation of Walker *et al.* using the treatment suggested by Son *et al.* and Roth *et al.*, with a reasonable expectation of success in producing the claimed invention.

Applicants respectfully disagree and traverse the rejection. In the present case, Son *et al.*, Roth *et al.*, and Walker *et al.* do not contain a suggestion or motivation to modify the references or to combine the references. *Second*, there is no reasonable expectation that the combination that the Examiner contemplates would succeed. Finally, Son *et al.*, Roth *et al.*, and Walker *et al.*, alone or in combination, do not teach or suggest all the claim elements.

As set out above, Son *et al.* and Roth *et al.*, alone or in combination, fail to disclose all of the elements of the claimed invention. Walker *et al.* also do not provide all of the elements of the claimed invention, alone or in combination with Son *et al.* and Roth *et al.* Walker *et al.* disclose a method for delivering a therapeutic agent in a liposome in an electrical field (see e.g., Abstract). Walker *et al.*, however, fail to disclose a liposomal formulation

wherein a foreign gene could be fully encapsulated such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C, as recited in independent claims 38 and 55.

In addition, Walker et al., fail to disclose that a foreign therapeutic gene can be successfully administered systemically as in independent claims 56 and 69. Furthermore, given the unpredictability of a delivery method being able to successfully deliver a gene (see page 5 of the Office Action) one of skill would not have a *reasonable* expectation that the combination of Son et al. and Roth et al. would result in successfully achieving the claimed invention.

Finally, Walker et al. fail to disclose that the foreign therapeutic gene can be administered in a lipid formulation comprising a cationic lipid and a lipid selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid as recited in independent claim 74. As such, Walker et al . do not teach all of elements of the claimed invention. Accordingly, a *prima facie* case of obviousness has not established. In view of the foregoing remarks, Applicants respectfully request that the rejection be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (415) 576-0200.

Respectfully submitted,

Eric J. Baude
Reg. No. P-47,413

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
EGW:ejb

WHAT IS CLAIMED IS:

1. (Canceled) A method of increasing the efficiency of transformation of cycling cells, said method comprising:

synchronizing cells at a first stage of the cell cycle; and

transforming said cells at a second stage of the cell cycle within about one cell cycle of said first stage with a nucleic acid that encodes a desired gene product.

2. (Canceled) A method of claim 1 wherein cells are synchronized by contacting said cells with an amount of a cell cycle synchronizer that is effective to synchronize cells at said first stage of the cell cycle.

3. (Canceled) A method of claim 2 wherein said cell cycle synchronizer synchronizes cells at a stage of the cell cycle when the nuclear membrane is substantially degraded.

4. (Canceled) A method of claim 1 wherein said cell cycle synchronizer synchronizes cells at late S phase.

5. (Canceled) A method of claim 1 wherein said cell cycle synchronizer synchronizes cells at the G_2/M phase boundary.

6. (Canceled) A method of claim 1 wherein said cell cycle synchronizer synchronizes cells at a stage other than M phase, and the nucleic acid accumulates in cells that have cycled to the G_2/M phase boundary.

7. (Canceled) The method of claim 1 wherein said cell cycle synchronizer is a vinca alkaloid.

8. (Canceled) The method of claim 1 wherein said cell cycle synchronizer is cisplatin.

9. (Canceled) The method of claim 1 wherein said cell cycle synchronizer is selected from the group consisting of taxol and taxolene.

10. (Canceled) A method of claim 1 wherein said first stage and said second stage are the same.

11. (Canceled) A method of claim 1 wherein said nucleic acid encodes a therapeutic gene and said therapeutic gene is foreign to the cell.

12. (Canceled) A method of claim 11 wherein the gene product of the therapeutic gene is toxic to the cell.

13. (Canceled) A method of claim 12 wherein the gene product of the therapeutic gene induces apoptosis.

14. (Canceled) A method of claim 1 wherein the nucleic acid is part of a lipid-nucleic acid particle.

15. (Canceled) A method of inhibiting the growth of cancer cells, said method comprising:

administering to a cancer patient an amount of a cell cycle synchronizer that is effective to synchronize cancer cells of said patient at a first stage of the cell cycle; and

administering to said cancer patient a nucleic acid that transforms cancer cells of said patient;

wherein the expression of said nucleic acid inhibits the growth of said cancer cells.

16. (Canceled) A method of claim 15 wherein said cancer cells are synchronized at a stage when the nuclear membrane is substantially degraded.

17. (Canceled) A method of claim 15 wherein said cell cycle synchronizer synchronizes the cell cycle at late S phase.

18. (Canceled) A method of claim 15 wherein said cell cycle synchronizer synchronizes the cell cycle at the G_2/M interphase.

19. (Canceled) A method of claim 15 wherein said cell cycle synchronizer synchronizes the cell cycle at a stage other than M phase, and the nucleic acid accumulates in cells when a plurality of cells exposed to the agent have cycled to the G_2/M interphase.

20. (Canceled) A method of claim 15 wherein said cell cycle synchronizer is a vinca alkaloid.

21. (Canceled) A method of claim 15 wherein said cell cycle synchronizer is cisplatin.

22. (Canceled) A method of claim 15 wherein said cell cycle synchronizer is selected from the group consisting of taxol and taxolene.

23. (Canceled) A method of claim 15 wherein said first stage and said second stage are the same stage of the cell cycle.

24. (Canceled) A method of claim 15 wherein said nucleic acid encodes a therapeutic gene.

25. (Canceled) A method of claim 24 wherein the therapeutic gene is foreign to the patient.

26. (Canceled) A method of claim 25 wherein the gene product of the therapeutic gene is toxic to said cells.

27. (Canceled) A method of claim 26 wherein the gene product of the therapeutic gene induces apoptosis of said cells.

28. (Canceled) A method of claim 15 wherein the nucleic acid is part of a lipid-nucleic acid particle.

29. (Canceled) A method of claim 15 wherein the nucleic acid is administered systemically.

30. (Canceled) A method of claim 24 wherein the therapeutic gene is expressed in said cancer cells.

31. (Canceled) A method of claim 30 wherein the therapeutic gene is HSV-TK and ganciclovir is also administered to said cancer patient.

32. (Canceled) A method of claim 15 wherein said cell cycle synchronizer is administered prior to administering said nucleic acid.

33. (Canceled) A method of claim 31 wherein said cell cycle synchronizer is administered at least 32 h prior to administering said nucleic acid.

34. (Canceled) A method of claim 31 wherein said cell cycle synchronizer is administered at least 48 h prior to administering said nucleic acid.

35. (Canceled) A method of claim 15 wherein said nucleic [cid] acid is administered prior to administering said cell cycle synchronizer.

36. (Canceled) A method of claim 15 wherein said nucleic acid is administered at least 32 h prior to administering said cell cycle synchronizer.

37. (Canceled) A method of claim 15 wherein said nucleic acid is administered at least 48 h prior to administering said cell cycle synchronizer.

38. (Once Amended) A method of enhancing the therapeutic effect of a foreign therapeutic gene administered to a patient, said method comprising the steps of

(a) administering a cell cycle blocking agent to said patient; and

(b) administering said foreign therapeutic gene to said patient within seven days of step (a), wherein said foreign therapeutic gene is fully encapsulated in a lipid formulation such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C.

39. (As filed) The method of claim 38 wherein step (b) is performed within 3 days of step (a)

40. (As filed) The method of claim 38 wherein step (b) is performed within 24 hours of step (a).

41. (As filed) The method of claim 38 wherein said foreign therapeutic gene is a plasmid.

42. (As filed) The method of claim 38 wherein said foreign therapeutic gene comprises a gene selected from the group consisting of genes encoding a cytokine, apoptotic protein, tumor suppressor, heat shock protein, immunogenic antigen, proteinase inhibitor, anti-angiogenic protein, suicide gene for use in GDEPT, ribozyme, antisense nucleic acid, viral protein and a toxin.

43. (As filed) The method of claim 38 wherein said foreign therapeutic gene is administered systemically.

44. (As filed) The method of claim 38 wherein said foreign therapeutic gene is administered locally or regionally.

45. (Canceled) The method of claim 38 wherein said foreign therapeutic gene is fully encapsulated in a lipid formulation such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C.

46. (As filed) The method of claim 38 wherein said cell cycle blocking agent is selected from the group consisting of DNA alkylating agents, DNA topoisomerase inhibitors, microtubule assembly inhibitors, microtubule disassembly inhibitors, DNA-cross linking agents, DNA-binding agents and nucleoside analogues.

47. (As filed) The method of claim 38 wherein said cell cycle blocking agent is selected from the group consisting of cyclophosphamide, etoposide, taxol, vincristine, cisplatin, doxorubicin and 5-fluorouracil.

48. (As filed) The method of claim 38 wherein said cell cycle blocking agent is in a liposome formulation.

49. (As filed) A method of claim 38 wherein said cell cycle blocking agent is administered prior to administering said foreign therapeutic gene.

50. (As filed) A method of claim 38 wherein said cell cycle blocking agent is administered at least 32 h prior to administering said foreign therapeutic gene.

51. (As filed) A method of claim 38 wherein said cell cycle blocking agent is administered at least 48 h prior to administering said foreign therapeutic gene.

52. (As filed) A method of claim 38 wherein said foreign therapeutic gene is administered prior to administering said cell cycle blocking agent.

53. (As filed) A method of claim 38 wherein said foreign therapeutic gene is administered at least 32 h prior to administering said cell cycle blocking agent.

54. (As filed) A method of claim 38 wherein said foreign therapeutic gene is administered at least 48 h prior to administering said cell cycle blocking agent.

55. (Once Amended) A method of enhancing the therapeutic effect of a cell cycle blocking agent, or of lowering the dosage of a cell cycle blocking agent required for a therapeutic effect, administered to a patient <u>having cancer</u> comprising the steps of:

(a) administering said cell cycle blocking agent to a patient; and

(b) administering a foreign therapeutic gene to said patient within seven days of step (a), <u>wherein said foreign therapeutic gene is fully encapsulated in a lipid formulation such that less than 5% of the gene is degraded after exposure of said formulation to 1 U DNAse I for 30 minutes in digestion buffer at 37°C</u>.

56. (New) A method of enhancing the therapeutic effect of a cell cycle blocking agent, or of lowering the dosage of a cell cycle blocking agent required for a therapeutic effect, administered to a patient having cancer comprising the steps of:

(a) administering said cell cycle blocking agent to a patient; and

(b) administering a foreign therapeutic gene to said patient within seven days of step (a), wherein said foreign therapeutic gene is administered systemically.

57. (New) The method of claim 38 wherein cells are synchronized by contacting said cells with an amount of said cell cycle blocking agent that is effective to synchronize cells at said first stage of the cell cycle.

58. (New) The method of claim 57 wherein said cell cycle blocking agent synchronizes cells at a stage of the cell cycle when the nuclear membrane is substantially degraded.

59. (New) The method of claim 38 wherein said cell cycle blocking agent synchronizes cells at late S phase.

60. (New) The method of claim 38 wherein said cell cycle blocking agent synchronizes cells at the G_2/M phase boundary.

61. (New) The method of claim 38 wherein said cell cycle blocking agent synchronizes cells at a stage other than M phase, and the nucleic acid accumulates in cells that have cycled to the G_2/M phase boundary.

62. (New) The method of claim 38 wherein said cell cycle blocking agent is a vinca alkaloid.

63. (New) The method of claim 38 wherein said cell cycle blocking agent is cisplatin.

64. (New) The method of claim 38 wherein said cell cycle blocking agent is selected from the group consisting of taxol and taxolene.

65. (New) The method of claim 38 wherein said first stage and said second stage are the same.

66. (New) The method of claim 38 wherein the gene product of the therapeutic gene is toxic to the cell.

67. (New) The method of claim 66 wherein the gene product of the therapeutic gene induces apoptosis.

68. (New) The method of claim 38 wherein the nucleic acid is part of a lipid-nucleic acid particle.

69. (New) A method of enhancing the therapeutic effect of a foreign therapeutic gene administered to a patient having cancer, said method comprising the steps of:

(a) administering a cell cycle blocking agent to said patient; and

(b) administering said foreign therapeutic gene to said patient within seven days of step (a), wherein said foreign therapeutic gene is administered systemically.

70. (New) The method of claim 69, wherein said cancer comprises a tumor.

71. (New) The method of claim 70, wherein said cell cycle blocking agent and said foreign therapeutic gene are administered distal to the site of the tumor.

72. (New) The method of claim 69, wherein said cell cycle blocking agent or said foreign therapeutic gene are administered intravenously.

73. (New) The method of claim 69, wherein said cell cycle blocking agent or said foreign therapeutic gene are administered intraperitoneally.

74. (New) A method of enhancing the therapeutic effect of a foreign therapeutic gene administered to a patient, said method comprising the steps of:

(a) administering a cell cycle blocking agent to said patient; and

(b) administering said foreign therapeutic gene to said patient within seven days of step (a), wherein said foreign therapeutic gene is administered in a lipid formulation comprising a cationic lipid and a lipid selected from the group consisting of an ATTA-lipid, a polyethylene glycol (PEG)-lipid derivative, and a ganglioside G_{M1}-modified lipid.

75. (New) The method of claim 74, wherein said (PEG)-lipid derivative is a PEG-ceramide.

76. (New) The method of claim 75, wherein said PEG-ceramide is a member selected from the group of PEG-Cer-C14, PEG-Cer-C20, and PEG-Cer-C8.

77. (New) The method of claim 74, wherein said lipid is present in an amount of from about 1% to about 20%.